

# Metabolism of Cadmium, Zinc and Copper in the Rat Kidney: The Role of Metallothionein and Other Binding Sites

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Studies were undertaken to determine the effect of host zinc deficiency upon the distribution of Cd, Zn and Cu between and within male rat kidney cytosol and unfractionated cell pellet. In the first experiment male rats were fed stock diets supplemented with 100 µg Cd/mL in the drinking water for 30 days. Then Cd-treated rats and controls were segregated into groups, which received semipurified diets adequate or deficient in zinc for 14 days. After this regimen there were comparable concentrations of total Cd and metallothionein bound Cd in cytosol and the supernatant of sonicated, unfractionated pellet on a µg Cd/g protein basis. Although less than 5% of cytosolic Cd is not bound to metallothionein (MT), 3-5 times as much non-MT Cd is present in the particulate fraction. The zinc-deficient (Zn-) dietary regime increases the non-MT Cd in the pellet. Quantitations were done of the Cu and Zn distribution in high molecular weight, superoxide dismutase, and metallothionein regions of the profiles of metals from Sephadex G-75 chromatography. In animals exposed to Cd and fed a zinc-normal (Zn+) diet, supernatant and pellet metal contents only change in the MT fraction. Similarly, zinc deficiency affects primarily the complement of metals bound to metallothionein: zinc is markedly decreased and Cu is lowered to a smaller extent. Cadmium is unchanged. Control kidney, unexposed to Cd, normally contains a substantial amount of Zn, CuMT. Two-week zinc deficiency greatly reduces MT-Zn and -Cu content without altering the metal content of other cellular pools. In a second experiment the kinetics of response of the concentration of Zn and Cu in plasma and kidney metallothionein to the imposition of a dietary zinc deficiency shows that Zn in these two compartments decreases rapidly and in parallel. Thus, both of these experiments point to metallothionein as a unique metalloprotein, which is metabolically sensitive to the nutritional state of the organism. In a third experiment, after rats were exposed to 20 µg Cd/mL drinking water for 30 days, basal synthesis of kidney metallothionein was not clearly enhanced in contrast to the response of kidney to the 100 µg Cd/mL, in which new synthesis was induced. Binding of Cd to MT occurs with the loss of Zn and Cu from the protein, thus, apparently reducing the number of binding sites on MT for Cu and Zn which are used for its normal unspecified functions in kidney.

## Introduction

The mammalian kidney is severely damaged by acute exposure to large quantities of cadmium (1,2). After such exposure, there is a time-dependent segregation of Cd among subcellular components of tissues such as liver and kidney (3,4). For

the first several hours Cd distributes itself among a variety of cellular constituents including the nucleus. Thereafter, the bulk of it is gathered up into a single protein in the cytosol. The cadmium which becomes bound to this protein, called metallothionein (MT), remains associated with it in the steady state, as induced synthesis of metallothionein balances its rate of biodegradation (5,6). Generally, induction of MT to bind Cd has been considered a protective response of cells to the presence of this toxic metal (7). Thus, in this model, kidney toxicity from acutely large doses of cadmium occurs either in the first hours after

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exposure before new metallothionein is synthesized or thereafter, only if the total binding capacity of induced MT for Cd is exceeded. In either case, it is the non-MT cadmium which is thought to be harmful.

It is of major environmental importance to consider the problem of chronic low level exposure of living system to cadmium. Relatively few studies have examined this situation with mammalian models. Despite some controversy, it seems clear that such exposure causes renal hypertension in experimental animals (8,9). Certainly, maternal exposure leads to greatly altered iron and copper metabolism in the neonate (10,11). Other studies show the induction of lung fibrosis and cardiotoxicity by similar low-level chronic exposure of rats to cadmium (11). The biochemical basis of these physiological perturbations is unknown. The difference between chronic and acute Cd exposure is that the massive reaction of Cd with cellular constituents before MT induction observed in acute exposure does not exist here. One only sees the slow accumulation of Cd in metallothionein. How, then, does Cd metabolism centering on metallothionein play a role in possible chronic toxicity? Metallothionein induction by Cd in liver and kidney results in steady-state increases in Zn and Cu as well as Cd in this protein. There also appear to be nutritional interactions of Cd and Zn. Thus, the approach of this study has been to examine the distribution of Cd, Zn and Cu in rat kidney as a function of the Cd and Zn status of the animal.

## Materials

Chow diet was purchased from Ralston-Purina. Semipurified diets used in the period following exposure of rats to 100  $\mu\text{g}$  Cd/mL drinking water and containing adequate and deficient levels of zinc were also obtained from Ralston-Purina. The control diet contains 20  $\mu\text{g}$  Zn/g diet and 15  $\mu\text{g}$  Cu/g diet. The deficient diet is the same except that its zinc content is less than 1  $\mu\text{g}$ /g. For the determination of the rate of loss of zinc from plasma and kidney metallothionein, a zinc-deficient diet from Ziegler-Brothers, Inc., Gardner, PA, was used. It contains less than 1  $\mu\text{g}$  Zn/g diet. Sephadex G-75 gel is a product of Pharmacia. Cadmium chloride used to expose animals to cadmium was reagent grade as were other chemicals.

## Methods

### Experimental Design

For the studies based on the exposure of ani-

mals to 100  $\mu\text{g}$  Cd/mL water, male Sprague-Dawley CD rats from Charles Rivers were housed individually in stainless steel cages with wire mesh floors. As summarized in Table 1, two groups of animals started the experiment, which were 3–5 months old and weighed about 340 g. Both were fed Purina lab chow for 30 days. One drank deionized water, the other, deionized water containing 100  $\mu\text{g}$  Cd/mL. At the end of this period each group was split in two. Each half received either a semipurified diet from Purina containing 20  $\mu\text{g}$  Zn/g (Zn + diet) or one with less than 1  $\mu\text{g}$  Zn/g [Zn(–) diet] for 14 days. All animals drank deionized water. After 2 weeks had elapsed, animals were anesthetized and bled by heart puncture. The heparinized blood was centrifuged to obtain plasma for metal analysis. Then the rats were sacrificed and several organs removed for study. Kidneys were weighed and in this experiment rapidly frozen and stored at  $-50^{\circ}\text{C}$ . During the experiment animals were weighed regularly and the dietary consumption monitored.

In a second related experiment, male King rats, (SD)BR, 2 months old and about 260 g in weight, were exposed for 30 days to 20  $\mu\text{g}$  Cd/mL of drinking water as they consumed Purina rat chow. Rats were sacrificed on day 31, 24 hr after removing Cd from the drinking water.

A third experiment was designed to follow the kinetics of depletion of Zn from plasma and kidney. King male rats, 3 months of age and weighing about 350 g were placed on a Zn(–) diet from Ziegler Brothers, together with drinking water containing 40  $\mu\text{g}$  Zn/mL for 24 hr to acclimate the animals to this diet. Then the animals were placed in clean, stainless steel cages, fed the Zn(–) diet and provided with glass-distilled water. At regular intervals thereafter, animals were sacrificed to obtain blood and kidney samples as described above. The concentration of Zn and Cu in plasma and their distribution among cytosolic proteins of the kidney were measured according to procedures outlined below.

### Sample Preparation

Kidneys were thawed, minced and added to 0.25 M sucrose containing a 5 mM 2-mercaptoethanol to retard oxidation of metallothionein (12). The mixture was mechanically homogenized and then centrifuged at 17,000g for 30 min. Supernatant and resuspended pellet were frozen at  $-50^{\circ}\text{C}$  until further use. At a later time the pellets were thawed and sonicated for 2 min at maximum power with a Branson sonicator. At no time did the temperatures of the samples rise above  $25^{\circ}\text{C}$ .

Clear supernatants were obtained after centrifugation of the sonicated pellets at 170,000g. They were stored at  $-50^{\circ}\text{C}$ . All glassware was pre-rinsed in deionized water and, in many cases, when deemed necessary, presoaked in 10%  $\text{HNO}_3$  prior to the water rinse.

## Metal Analyses

Samples of cytosol or sonicated pellet material were placed in  $2 \times 40$  cm columns of Sephadex G-75 and eluted with 50 mM Tris Cl buffer, pH 7.8. Column fractions were analyzed for Zn, Cu, Fe and Cd by flame atomic absorption spectrophotometry. Protein content of the supernatants was measured by the Lowry method (13).

## Results

### Macroscopic Effects of Zn(+) and Zn(-) Diets on Rats Pretreated with Cadmium

With the protocol described above for Experiment 1, the sequential exposure of rats to 100  $\mu\text{g}$  Cd/mL drinking water and dietary zinc deficiency produced the gross effects upon male rats shown in Table 1. After 30 days of cadmium intake, animals had grown significantly less than unexposed controls. Organ weights did not show the difference (groups A and B). During the next 2 weeks on semipurified diets, the two groups on the zinc-deficient diet lost weight while the others gained. This is a reflection of the sensitivity of growing rats to zinc deficiency. During these 2 weeks there was significant decrease in organ weights in group F relative to group D controls and the group E rats. In fact, in comparison with

the two groups sacrificed after 4 weeks, the impression is that the Cd-treated animals had lost organ weight during the period of zinc deficiency.

In the course of the 2-week regimens in semipurified diets, both zinc-deficient groups ate somewhat less diet than their zinc-sufficient counterparts. Whole animal growth in group E now paralleled the controls of group C. As expected, zinc deficiency impairs weight gain to about the same extent in both D and F groups of animals. In several long-term experiments these kinds of differential changes in organ weights were variably found, suggesting that the experiment places the animals within a sensitive range for Cd-Zn interactions.

### Cd, Zn and Cu Distribution in Kidney Cytosol and Pellet

The control, group C rat kidney cytosol has the typical G-75 Sephadex profile shown in Figure 1. A similar profile for the supernatant of sonicated kidney pellet from the same animal is given in Figure 2. Both cytosol and the whole particulate fraction contain a Zn, Cu binding protein, eluting at 10,000 daltons, taken to be metallothionein. The Zn/Cu ratios in the bands of MT are 1:1 and 1:3.5 for cytosol and pellet, respectively. These differences alone argue that the pellet protein is not due to contaminated cytoplasmic protein in the preparation. For the purposes of the metal quantitation, described in Table 2, the profiles were divided into three sections (I-III), as shown in Figure 3: fraction 1 represents high molecular weight protein; II, the superoxide dismutase region; and III, the metallothionein part of the

Table 1. Weight changes of rats exposed sequentially to Cd and zinc deficiency.

Group <sup>a</sup>	Metal exposure			Animal weight change, g		Consumption of diet, days 30-44, g	Organ weights on day of sacrifice, g			Plasma Zn, $\mu\text{g/mL}$
	Days 1-30, Cd	Days 31-44		Day 1-30	Day 30-44		Liver	Kidney	Heart	
A(4)				101 $\pm$ 15 <sup>c</sup>			17.6 $\pm$ 1.0	2.98 $\pm$ 0.17	1.19 $\pm$ 0.06	
B(4)	X <sup>b</sup>			82 $\pm$ 7			17.2 $\pm$ 1.9	2.97 $\pm$ 0.18	1.16 $\pm$ 0.07	
C(5)		X		100 $\pm$ 11 <sup>d</sup>	30 $\pm$ 8 <sup>†</sup>	175 $\pm$ 13	16.6 $\pm$ 1.2	2.70 $\pm$ 0.09	1.25 $\pm$ 0.02	1.41 $\pm$ 0.08 <sup>†</sup>
D(6)			X	105 $\pm$ 9	-9 $\pm$ 4 <sup>†</sup>	139 $\pm$ 12	16.8 $\pm$ 0.4 <sup>†</sup>	2.74 $\pm$ 0.08*	1.30 $\pm$ 0.04*	0.97 $\pm$ 0.06 <sup>†</sup>
E(5)	X	X		77 $\pm$ 6 <sup>e</sup>	26 $\pm$ 8 <sup>†</sup>	167 $\pm$ 6	16.1 $\pm$ 0.6	* 2.94 $\pm$ 0.03 <sup>†</sup>	1.27 $\pm$ 0.06	* 1.50 $\pm$ 0.34*
F(6)	X		X	90 $\pm$ 5	-18 $\pm$ 6 <sup>†</sup>	123 $\pm$ 19	14.0 $\pm$ 0.6 <sup>†</sup> ,*	2.32 $\pm$ 0.11*, <sup>†</sup>	1.13 $\pm$ 0.04*,*	0.91 $\pm$ 0.14*

<sup>a</sup>Capital letters designate the group as defined by its metal exposure on days 1-30 and 31-44. During the first 30 days all animals were fed stock chow. Number of animals in group given in parentheses.

<sup>b</sup>X indicates exposure to Cd and the type of semipurified feed.

<sup>c</sup>Mean  $\pm$  standard error of the mean. All pairs of data in columns were compared for statistically significant differences by the *t*-test for unpaired variates:  $p < 0.05$ (\*),  $p < 0.01$ (<sup>†</sup>). Pairs of symbols in the same column denote sets of data in which significant differences were observed.

<sup>d</sup>Mean  $\pm$  SEM for combined groups C and D, 103  $\pm$  7 g, which is significantly different from combined average for E and F ( $p < 0.05$ ).

<sup>e</sup>Mean  $\pm$  SEM for combined groups E and F; 84  $\pm$  4 g.

metal distribution. Low molecular weight metal elutes beyond metallothionein.

The imposition of the zinc-deficient condition upon Zn(+) controls depletes cytosol and pellet of

metallothionein zinc and copper (Fig. 1 and Table 2). From a comparison of average data from a number of animals in Table 2, zinc deficiency causes the loss of zinc from MT, leaving levels of the metal barely above detection limits. Interestingly, copper is lost as well, but to a somewhat smaller, marginally significant, extent. Conspicuously, in cytosol it is only the MT band which responds to zinc deficiency. Fractions I and II exhibit no significant changes in Zn content due

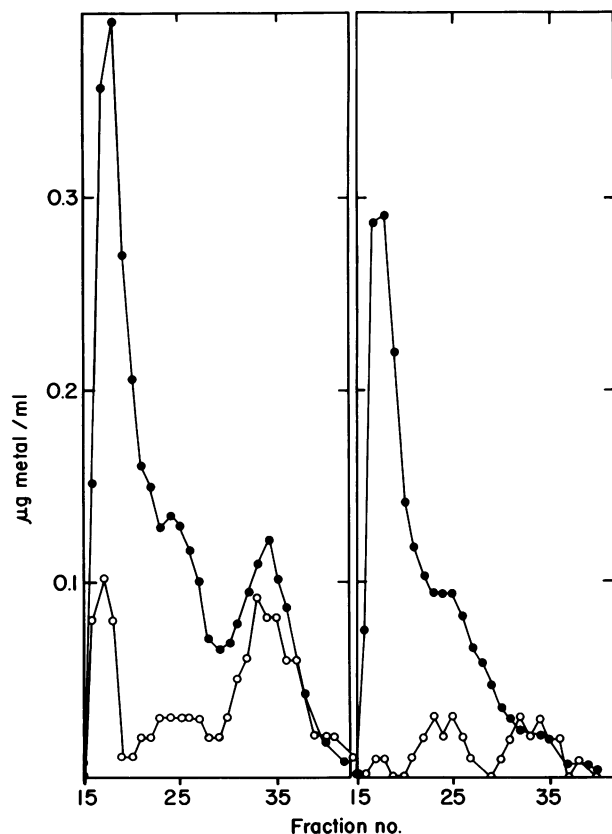


FIGURE 1. G-75 Sephadex chromatographic profile of cytosol from kidney not exposed to Cd: (left) cytosol of Zn(+) kidney containing 16.4 mg protein /mL; (right) cytosol from Zn(-) kidney, 18.2 mg protein/mL; (●) Zn, (○) Cu.

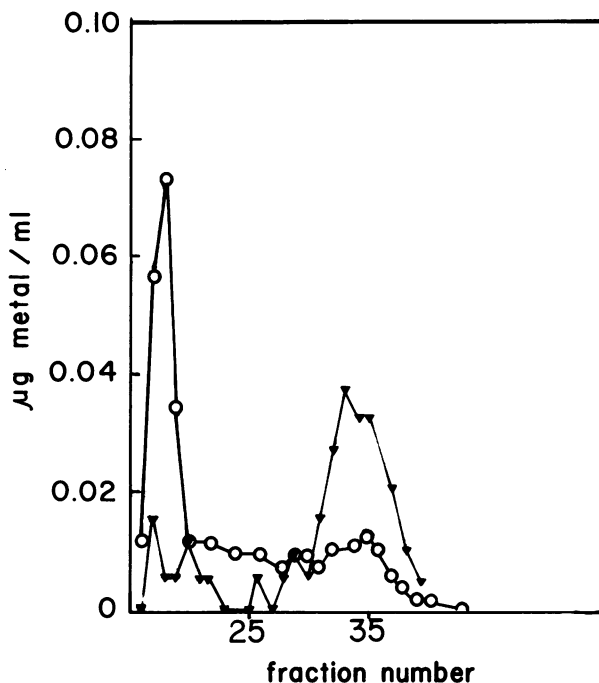


FIGURE 2. G-75 Sephadex chromatographic profile of pellet sonicate from kidney not exposed to Cd, 2.1 mg protein/mL: (○) Zn, (▼) Cu.

Table 2. Zn, Cu, Cd distribution in kidney cytosol.

Metal	Group <sup>a</sup>	Metal concn in various chromatographic fractions, µg/g protein <sup>b</sup>		
		I (High MW)	II (Superoxide dismutase)	III (MT)
Zn	C (5)	57.2 ± 5.3	25.0 ± 2.5	27.2 ± 2.9 <sup>†,†</sup>
	D (6)	54.9 ± 7.3	27.4 ± 1.7	12.2 ± 3.2 <sup>†</sup>
	E (5)	52.0 ± 3.2	24.6 ± 1.4	67.5 ± 6.8 <sup>†</sup> <sup>†</sup>
	F (6)	61.8 ± 8.3	26.8 ± 1.8	31.9 ± 3.2 <sup>†,†</sup>
Cu	C (5)	6.3 ± 1.5	10.8 ± 1.0 <sup>*</sup>	24.0 ± 2.4 <sup>°,†</sup>
	D (5)	3.1 ± 1.3	7.4 ± 0.4 <sup>*</sup>	15.0 ± 4.2 <sup>°</sup> <sup>*</sup>
	E (5)	4.5 ± 1.1	9.0 ± 1.1	43.9 ± 5.1 <sup>†</sup>
	F (6)	3.3 ± 1.0	9.0 ± 1.0	33.9 ± 5.8 <sup>*</sup>
Cd	E (5)		2.9 ± 1.2	53.9 ± 3.8
	F (6)		3.8 ± 0.8	63.2 ± 5.6

<sup>a</sup>Dietary regimen of groups defined in Table 1. Number of animals in parentheses.

<sup>b</sup>Means ± standard error of the mean. All data for given metal and chromatographic fraction were compared by the *t* test for unpaired variates to find statistically significant differences: *p* < 0.1 (°), *p* < 0.05 (\*), *p* < 0.01 (†). Pairs of symbols in the same column denote sets of data in which significant differences were calculated.

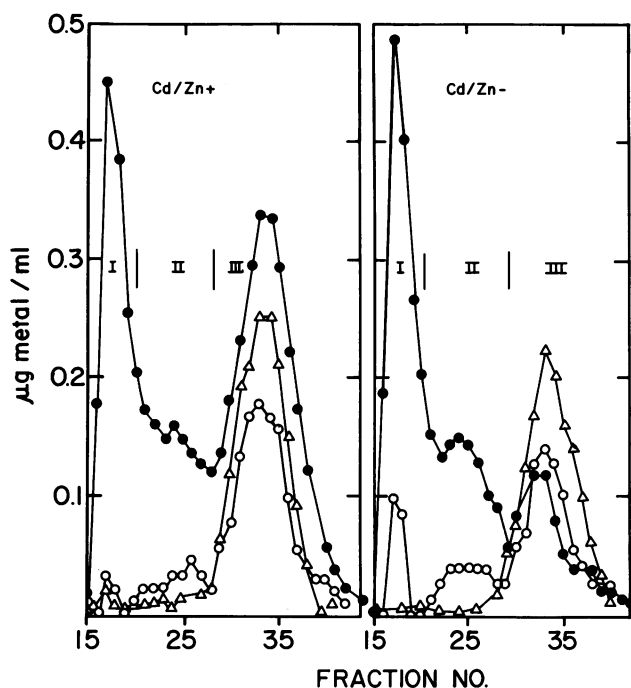


FIGURE 3. G-75 Sephadex chromatographic profile of kidney cytosol from animals exposed to 100 mg Cd/mL drinking water: (left) cytosol from Zn(+) kidney, 21.4 mg protein/mL; (right) cytosol from Zn(-) kidney, 21.4 mg protein/mL; (●) Zn, (○) Cu, (△) Cd.

to Cd exposure. However, fractions II of cytosol and fractions I and II of pellet appear to have lower Cu levels in zinc-deficient animals.

Metal distribution studies of cadmium-exposed kidneys reveal the typical cytosolic profile of Cd in which greater than 95% of the cadmium is bound to MT (Fig. 3 and Table 2). There has been induction of new MT protein above the basal control level. New protein accommodates not only Cd but also additional Zn and Cu. The presence of Cd has no effect upon the rest of the profiles of Zn and Cu.

The pellet also contains cadmium, the majority of which is bound to metallothionein or a MT-like protein. In fact, on a protein basis, there is a larger concentration of Cd in the sonicated supernatant of the total particulate fraction than there is in cytosol (Tables 2 and 3). Moreover, a much smaller fraction of Cd is MT-bound according to these studies. Only about 70–80% is in the MT region of the profile while the rest is distributed over other fractions. An elevated but not statistically significant concentration of non-MT Cd exists in the pellet supernatants of groups E and F animals. The isolation of pellet and supernatant in the presence of 2-mercaptoethanol minimizes sulfhydryl oxidation. The relative insensitivity of

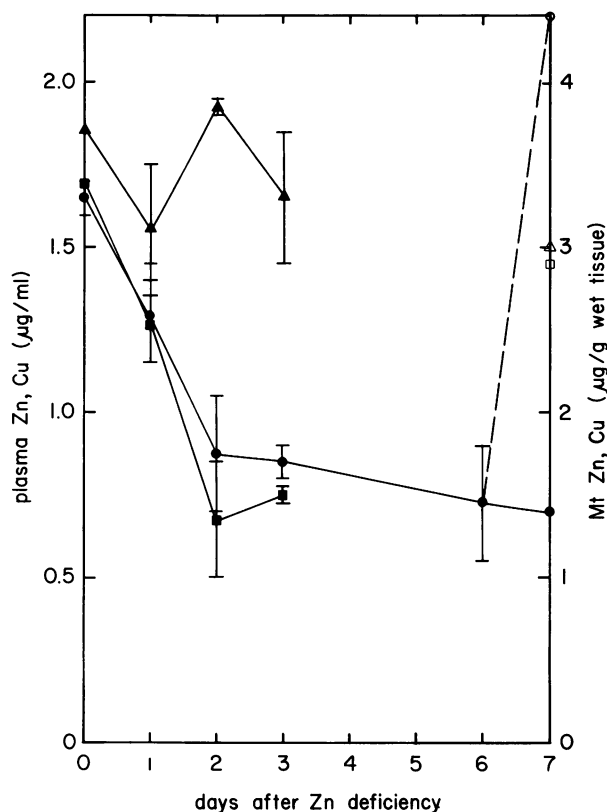


FIGURE 4. Kinetics of change in concentration of Zn and Cu in plasma and kidney metallothionein: (●) plasma Zn after imposition of Zn deficiency; (○) plasma Zn 22 hr after addition of Zn to water; (■) MT-Zn and (▲) Cu after Zn deficiency; (□) MT-Zn and Cu (△) 22 hr after addition of Zn to water. Horizontal bars about the means represent actual data points.

Cd-MT to oxidation and the clear differences between cytosol and pellet in metal distribution argue that the enhanced amount of non-MT Cd is real and not an artifact of isolation (12). Again, as in cytosol, the Cu and Zn profiles differ only in the MT band, which contains elevated levels of Cu and Zn along with Cd.

### Changes in Plasma and Kidney Zn during Zn Deficiency

Kidney is unusual among mature rat tissues in that it contains a large concentration of Zn, Cu metallothionein in the absence of stress or elevated metal exposure. Presumably, therefore, it has a normal physiological role to play in kidney metabolism. Having noted the specific loss of Zn from kidney metallothionein after 2 week zinc deficiency, it was of interest to determine the kinetics of this effect. The results in Figure 4 show that about 50% of the plasma Zn is labile

and is lost progressively over 48 hr following imposition of a Zn(-) diet and water on a set of rats. Metallothionein zinc declines over this same period and thus appears to be tightly coupled to mobilizable plasma zinc. This conclusion is strengthened by the observation that within 24 hr of the addition of Zn to the drinking water of 7 day Zn(-) animals, both plasma and kidney MT zinc have risen to normal or above normal levels. It is striking that although Cu is lost from MT after the 14-day zinc deficiency (Table 2), it is clearly lost at a slower rate than zinc. The lack of change in MT-Cu was mirrored in the plasma Cu level, which remained constant at control levels ( $1.0 \pm 0.03 \mu\text{g Cu/mL}$ , mean  $\pm$  standard error for 12 samples) over the course of the experiment.

### Localization of Cd in Kidney Cytosol after Low Level Exposure

The recognition that kidney metallothionein zinc reacts rapidly to changes in plasma levels of zinc caused by zinc deficiency suggests that it may play a role in metal homeostasis in the

kidney and that the binding of Cd to MT might perturb its normal function. Chronic exposure of animals to  $100 \mu\text{g Cd/mL}$  drinking water induced extra MT synthesis and increased the total number of binding sites for Zn and Cu in kidney (Tables 2 and 3). To see if a lower Cd level would simply replace zinc in binding sites of preexistent MT instead of inducing new synthesis, King rats were given  $20 \mu\text{g Cd/mL}$  drinking water for 30 days. Table 4 summarizes the Cd, Zn and Cu content of cytosolic MT in several groups of animals. Although the sample size was small, there was no statistically significant evidence that Cd increased the total metal content of MT. Instead, the presence of Cd in MT was accompanied by compensating small decreases in Zn and Cu content. If these animals had responded as those getting  $100 \mu\text{g Cd/mL}$  in Table 2, an expected elevation in metal content of MT would include  $0.4 \mu\text{g Cu}$  and  $0.8 \mu\text{g Zn}$  per  $1.0 \mu\text{g Cd}$  to yield a total metal content of about  $13.4 \mu\text{g/g}$ , nearly twice that observed in these kidneys. Thus, this experiment suggests that low level exposure of kidney to Cd leads to the reduction of binding

Table 3. Zn, Cu, Cd distribution in kidney pellet.

Metal	Group <sup>a</sup>	Metal concn in various chromatographic fractions, $\mu\text{g/g protein}^b$		
		I (High MW)	II (Superoxide dismutase)	III (MT)
Zn	C (4)	$77.5 \pm 16.4$	$33.6 \pm 9.2$	$20.2 \pm 12.2^*$
	D (3)	$65.7 \pm 6.1$	$23.6 \pm 2.1$	$6.2 \pm 7.2$
	E (5)	$99.0 \pm 14.3$	$28.0 \pm 3.9$	$60.1 \pm 5.1^{*,\dagger}$
	F (5)	$72.3 \pm 8.7$	$24.1 \pm 4.1$	$15.8 \pm 5.5^\dagger$
Cu	C (4)	$35.1 \pm 3.0^\dagger$	$31.9 \pm 3.6^\dagger$	$102 \pm 11^\dagger, \dagger$
	D (3)	$15.0 \pm 2.5^\dagger$	$9.8 \pm 1.1^\dagger, *$	$30 \pm 2^\dagger$
	E (5)	$32.5 \pm 10.5$	$41.6 \pm 8.4$	$212 \pm 26^\dagger, *$
	F (5)	$24.9 \pm 4.2$	$36.9 \pm 8.3^*$	$125 \pm 6^*$
Cd <sup>c</sup>	E (5)	$13.6 \pm 5.6$	$17.0 \pm 8.0$	$132 \pm 20$
	F (5)	$13.9 \pm 3.3$	$31.0 \pm 3.4$	$139 \pm 10$

<sup>a</sup>Dietary regimen of groups defined in Table 1. Number of animals in parentheses.

<sup>b</sup>Mean  $\pm$  standard error of the mean. All data for a given metal and chromatographic fraction were compared by the *t* tests for unpaired variates to find statistically significant differences:  $p < 0.05$  (\*),  $p < 0.01$  (†).

<sup>c</sup>Low molecular weight Cd, appearing in fractions beyond metallothionein: group E,  $1.5 \pm 1.5$ ; group F,  $5.8 \pm 2.5$ .

Table 4. Metal content of cytosolic metallothionein of rats exposed to  $20 \mu\text{g Cd/mL}$  drinking water.

Group (n) <sup>a</sup>	Metal content, $\mu\text{g/g kidney wet weight}^b$			
	Zn	Cu	Cd	Total
Control (3)	$3.20 \pm 0.32$	$3.59 \pm 0.50$		$6.79 \pm 0.65$
Cd-treated (3)	$2.53 \pm 0.37$	$2.72 \pm 0.34$	$2.20 \pm 0.25$	$7.79 \pm 0.24$
Cd-treated <sup>c</sup>	5.8	5.4	2.2	13.4

<sup>a</sup>Number of animals in parentheses.

<sup>b</sup>Mean  $\pm$  standard error of the mean. It is assumed that Charles Rivers and King rats respond similarly to cadmium. None of the differences in Zn or Cu content between control Cd-treated animals was statistically significant by the *t* test for unpaired variates at the  $p < 0.05$  level.

<sup>c</sup>Hypothetical metal distribution, assuming induction of Zn,Cu,CdMT as seen in Table 2.

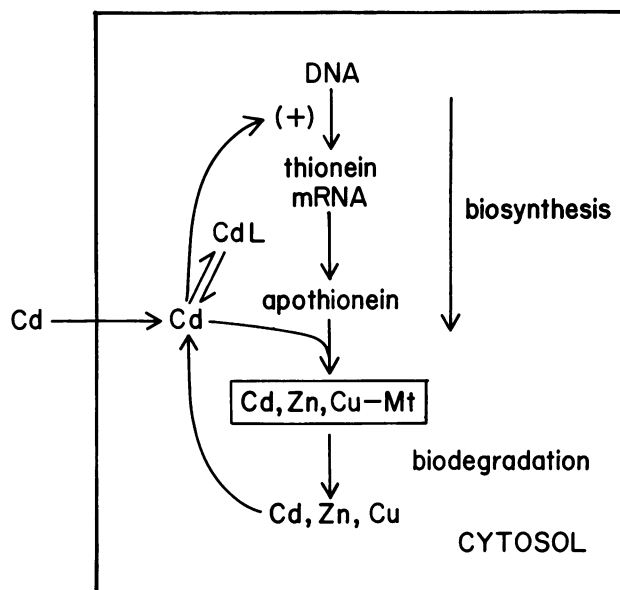


FIGURE 5. Model for the metabolism of cadmium in kidney.

sites of MT for normal Zn and possibly Cu metabolism.

## Discussion

The general picture of acute Cd toxicity to the kidney can be described in terms of Figure 5, which summarizes now familiar aspects of Cd metabolism. When a large concentration of Cd enters the kidney after injection of Cd or Cd-MT, it binds to a variety of macromolecular ligands (L), and causes severe damage to the organ (1,2). Over the course of several hours it also induces sufficient synthesis of apothionein to bind up almost all of the cytosolic Cd into an inert form which is innocuous for the kidney. Further direct toxicity occurs only if uptake and binding of Cd to CdL exceeds the ongoing induction rate or if the concentration of Cd,Zn,Cu-MT is so large that biosynthesis of apothionein cannot keep pace with biodegradation and the levels of CdL increase. Another route of toxicity might be the alteration in zinc and copper metabolism of kidney related in an unspecified way to metallothionein as Cd binds to the protein. An enormous amount of effort has gone into defining and validating this model of Cd, Zn and Cu metabolism and interaction (14). While it explains short-term acute toxicity, it suggests that chronic exposure, which never exposes the kidney to high concentrations of Cd, is innocuous until, after very long periods, the concentration of Cd,Zn,Cu-MT becomes so high that the biodegradation rate ex-

ceeds the rate of biosynthesis of apothionein, and non-MT-CdL complexes build-up.

The present studies seek to enlarge conceptually upon this model. First, it is clear that significant amounts of Cd exist in the particulate fraction of kidney. Because cytosol is the largest cell compartment, most of the Cd appears in this fraction. However, if expressed as per unit weight protein, the concentration of Cd in the whole pellet is comparable to that in cytosol. In work to be published elsewhere, it is shown that membrane, mitochondrial and postmitochondrial fractions all contain Cd. Interestingly, the amount of non-MT-Cd in pellet is much larger than in cytosol. Thus, since many critical biochemical activities occur in organelles, one needs to look for sites potentially sensitive to Cd in the organelle compartments of kidney.

The results presented here resemble in some respects information published recently (15, 16) on the subcellular distribution of Cd in kidney after exposure of rats to large amounts of Cd for months. Attention was focused on high molecular weight non-MT-Cd which appears over time. In fact, if interpreted correctly by the authors, Sato and Nagai (15) show that organelle fractions contain large concentrations of Cd, on a per milligram basis, throughout the experiments. Naturally, the duration and extent of exposure produces kidney levels of MT many times those of Table 2. However, as demonstrated here, such high level exposure is not necessary to produce non-MT-Cd.

There may be an elevation of non-MT-cadmium in the kidney pellets of zinc-deficient animals relative to Zn normal controls. This was not accompanied by measurable changes in MT-bound cadmium in cytosol or pellet, even though marked reduction in MT-Zn occurred during zinc deficiency. Thus, the binding of Cd to metallothionein does not require the presence of normal, steady-state levels of zinc in the protein.

An adequate supply of zinc to the kidney does appear to be necessary to sustain the long-term, steady-state binding of Cu to metallothionein. Over the course of 2 weeks, both Zn and Cu are lost from metallothionein during zinc deficiency (Tables 2 and 3). Interestingly, the loss of zinc from Cd,Zn,Cu-MT does not deplete the protein of Cu in cytosol as much as in pellet. Bremner also found (17) that pregnant female rats or ones receiving progesterone lose copper as well as zinc from kidney MT during zinc deficiency. Whether or not a hormonal stimulus is necessary to obtain the effect in females, the present results show that this response is also seen in normal male

rats (17). In addition, as described below, the kinetics of loss of Cu from MT is much slower than the rate of change in MT-Zn concentration after the imposition of dietary zinc deficiency.

The final points about the experiment center on the effects of cadmium and zinc deficiency on Cu and Zn distribution in kidney. Tables 2 and 3 show clearly that the only effect of Cd is to elevate MT-Zn and Cu. Other components of cytosol and pellet are unaffected at this level of analysis. The effect of zinc deficiency is also focused in metallothionein. Despite the 2-week period of zinc deficiency, during which kidney proteins are expected to turn over several times, other fractions of zinc do not lose zinc. Thus, it is Zn,Cu-MT which responds to the nutritional state of the animal.

The sensitivity of kidney Zn,Cu-MT to the zinc status of the host draws attention to the question of the normal function of this protein. This question is underscored by unpublished findings that liver Cd,Zn-MT is rather insensitive to zinc-deficient conditions, in contrast to the kidney protein. The results summarized in Figure 4 underscore the remarkably close relationship of plasma and kidney metallothionein levels of zinc. One may suggest that this linkage reflects a role for MT in the filtration of blood plasma by the kidney. Thus, MT might serve as the primary metal exchange site to move zinc from the glomerular filtrate through the proximal tubule and back into the blood. The finding that metallothionein is localized in renal tubular epithelium lends support to this view (18,19). A striking feature of Figure 4 is the independence of Zn and Cu in MT in response to zinc deficiency. Either Zn and Cu bind to different molecules of MT or, after the loss of zinc from the mixed metal protein, an all-copper species is formed and persists.

The recognition that kidney normally contains a Zn,Cu metallothionein, which is involved in metal metabolism, leads to the question whether cadmium, entering the kidney and binding to MT, can disrupt the normal functions of this protein. If this occurs, the presence of Cd in the protein might either reduce the functional activity of Zn and Cu in the two metal clusters of metallothionein or reduce the number of binding sites available for zinc and copper on the protein (20). Given the recent evidence that a calf liver Zn,Cu-MT segregates the metals between the clusters, it is reasonable that rat kidney MT, binding about equal amounts Zn and Cu, also has zinc bound preferentially in the four-metal cluster and copper in the three-metal cluster (21). In rats drinking 100 µg Cd/mL water, cytosolic MT binds Zn, Cu and Cd in a proportion of 1.0:0.66:0.49 in

contrast to the native protein with a Zn, Cu ratio of 1:0.9 (Table 2). In the net induced protein, the Zn, Cu, Cd proportion is 1:0.5:0.8. How these metals are arranged in the metal clusters is a particularly interesting question. Assuming the concept that homogeneous metal clusters naturally exist in metallothionein is correct, then a complex mixture of cluster combinations must be formed during inductive synthesis of Cd,Zn,Cu-MT in kidney (22). If, in fact, Cd is segregated away from Zn or Cu, then it is unlikely that it will affect direct MT-Zn or Cu metabolism, which possibly depends on direct metal transfer reaction between ligands, such as



because no intracluster Cd-Zn or Cd-Cu interaction would exist to modify reactivity (23). However, if metal movements depend on protein turnover, the introduction of Cd into the protein might significantly retard that process, for the  $t_{1/2}$  of Cd-MT turnover is three to five times as long as that for Zn or Cu-MT (14).

The exposure of kidney to lower levels of Cd as described in Table 2 has the possibility of reducing the number of binding sites in MT for Zn and Cu and, thereby, of inhibiting the normal function of MT in kidney. Further studies are necessary to confirm and extend this finding to be certain that new, net synthesis of MT has not occurred under these conditions. If the present results are sustained, then one must look carefully at the effects of Cd which may occur in kidney and other tissues below thresholds for induction of new thionein, for in the present view it is only the induction of thionein and its irreversible steady-state binding of Cd which protects cells from this metal.

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